

bition of oxidized form of SDH by malonate (malonate was added before quinone) activates respiration rate. Double inhibitor titration method showed that in presence of DTD inhibition of SDH is accompanied by shift of the limiting stage of Q-cycle from i-center to o-center. According to suggested model the electron fluxes from SDH and DTD compete with each other in i-center of Q-cycle resulting in super reduction of i-center. Thus partial inhibition of the one of this fluxes yields oxidation of i-center and leads to increasing of respiration rate.

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S13.44 Role of THE NapGH menaquinol dehydrogenase complex in *Wolinella succinogenes* nitrate respiration

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Many membrane-integral quinone-reactive enzyme complexes that are part of both eukaryotic and prokaryotic respiratory electron transport chains contain one or more haem *b* molecules. In recent years, however, a variety of novel proteins devoid of haem *b* emerged that are proposed to fulfil a similar function in anaerobic respiratory systems of various bacteria, e.g. members of the *c*-type cytochrome family NapC/NrfH and iron-sulfur proteins such as NapH. The *napH* gene is frequently present in gene clusters encoding components of the bacterial periplasmic nitrate reductase system. It is predicted to contain four transmembrane segments and to form a quinol oxidising complex with another iron-sulfur protein, NapG. We show here that NapH and NapG of the nitrate-respiring ϵ -proteobacterium *Wolinella succinogenes* indeed form a membrane-bound complex that mediates electron transfer from menaquinol to nitrate. The NapG subunit is located at the periplasmic side of the membrane where it acts as an electron transfer adapter protein that specifically donates electrons to the nitrate reductase NapA. A NapH homologue, NosH, is also able to form a functional complex with NapG. Deletion of either *napH* or *napG* almost abolished growth by nitrate respiration. The possible function of the essential cytoplasmic poly-cysteine clusters of NapH in the bioenergetics of nitrate respiration and/or in redox-driven enzyme maturation is discussed.

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S13.45 *Chlamydomonas reinhardtii* mitoproteome adaptation in response to inactivation of the energy-dissipating alternative oxidase 1 by RNA interference

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The mitochondrial alternative oxidase (AOX) is an ubiquinol-oxygen oxidoreductase which catalyses ubiquinol oxidation by molecular oxygen. Thus AOX competes for electrons with the cytochrome pathway, generating an electron partitioning and decreases the oxidative phosphorylation yield. AOX from the unicellular green alga *Chlamydomonas reinhardtii* is encoded by two genes, the AOX1 gene

being much more transcribed than AOX2. In addition, the expression of the AOX1 gene is down-regulated by ammonium and stimulated by nitrate. In this work, we performed a comparative proteomics approach (2D-DIGE) to study the effects of the inactivation of AOX1 by RNA interference on the mitochondrial proteome of *Chlamydomonas reinhardtii* cultivated on nitrate. Our results indicate that 88 protein spots are statistically up or down-regulated in our experimental conditions. Interestingly, observed up and down-regulations were related to proteins involved in protection against ROS and RNS. Moreover, other important enzymes of the main mitochondrial metabolic pathways (Krebs cycle, amino-acid metabolism and several subunits of the mitochondrial respiratory chain complexes) were also regulated indicating the important impact of the alternative oxidase expression in oxidative stress defence as well as in metabolic turnover.

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(S14) Mitochondria and ageing symposium lecture abstracts

S14/1 Cardiolipin as an oxidative target in cardiac mitochondria in the aged rat

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The aged heart sustains greater injury during ischemia (ISC) and reperfusion (REP) compared to the adult heart. In the Fischer 344 (F344) rat, aging decreases oxidative phosphorylation and complex III activity increasing the production of reactive oxygen species in interfibrillar mitochondria (IFM) located among the myofibrils. In the isolated, perfused 24 month old elderly F344 rat heart 25 min. of stop-flow ISC causes additional damage to complex III, further decreasing the rate of OXPHOS. We did not observe further progressive mitochondrial damage during REP. We next asked if ISC or REP increased oxidative damage within mitochondria of the aged heart. Cardiolipin (CL) is a phospholipid unique to mitochondria consisting predominantly of four linoleic acid residues (C18:2). Following ISC and REP in the aged heart, there is a new CL species containing three oxygen atoms added to one linoleic residue. ISC alone was sufficient to generate this new oxidized molecular species of CL. Based upon oxidative damage to CL, complex III activity, and oxidative phosphorylation, mitochondrial damage thus occurs in the aged heart mainly during ISC, rather than during REP. Mitochondrial damage during ischemia sets the stage for mitochondrial-driven cardiomyocyte injury during reperfusion in the aged heart. Supported by: NIH PO1 AG15885.

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S14/2 Mitochondrial volume regulation by a redox switch on the adenine nucleotide translocase

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Mitochondrial volume regulation plays an important role in the control of oxidative phosphorylation and protection against cell injury;

however, little is known about which proteins mediate ion transport across the mitochondrial inner membrane. Here, we uncover a novel mechanism regulating mitochondrial volume that depends on glutathione levels, the adenine nucleotide translocase, and substrate metabolism. Addition of 3 mM reduced (GSH), but not oxidized (GSSG; up to 0.3 mM), glutathione to isolated guinea pig heart mitochondria, in the absence of exogenous substrates, triggers a dramatic, switch-like, transient contraction and sustained swelling response involving matrix expansion and remodeling of inner membrane cristae. Remarkably, mitochondrial swelling could be reversed by tricarboxylic acid (TCA) cycle intermediates with a preferred selectivity of the substrate, as follows: citrate = isocitrate > succinate > malate > oxaloacetate > glutamate. Preincubation with the six TCA cycle intermediates either blunted or prevented the acute swelling response to 3 mM GSH depending on the substrate. The GSH-induced swelling occurred in parallel with acute NAD(P)H oxidation. Adding ADP and bongkrekic acid before GSH completely blocked the swelling response, or contracted mitochondria pre-swollen with GSH, indicating the adenine nucleotide translocase (ANT) was acting as a redox-sensitive pore. The response was insensitive to the permeability transition pore inhibitor cyclosporine A or the inner membrane anion channel inhibitor 4'-chlorodiazepam (4-Cl-DZP). The findings highlight an important interaction between the glutathione and pyrimidine nucleotide pools that participates in mitochondrial volume regulation by changing the conformation of the adenine nucleotide translocase.

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S14/3 Targeting molecules to mitochondria

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Mitochondrial function and dysfunction contributes to a range of important aspects of biomedical research. Consequently there is considerable interest in developing approaches to modify and report on mitochondria in cells and in vivo. One approach has been to target bioactive molecules to mitochondria by conjugating them to lipophilic cations. Due to the large mitochondrial membrane potential, the cations are accumulated within mitochondria inside cells. This approach has been used to develop mitochondria-targeted antioxidants that selectively block mitochondrial oxidative damage and prevent some types of cell death and also to develop probes of mitochondrial function. Here we outline some of the background to the development of these compounds.

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(S14) Mitochondria and ageing symposium abstracts (poster and raised abstracts)

S14.4 Random mtDNA mutations cause respiratory dysfunction through failure in complex assembly

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Mice with defective proofreading in the mitochondrial specific DNA polymerase polg, (mtDNA mutator mice) have increased mito-

chondrial DNA mutation rate and a premature aging phenotype, including hair loss, cardiomyopathy, early loss of fertility in females, anaemia, kyphosis, osteoporosis and progressive hearing loss. We set out to find out what effect the mutations would have on the mitochondria. Previously we have shown that there is a progressive loss of activity in respiratory chain complexes that are partially encoded by mtDNA (I, III, IV and V). We now show that isolated mitochondria from these mice display reduced oxidative capacity. Western blot analysis of single protein subunits shows that there is a reduction in levels of COX II and IV, while levels of all other analysed protein subunits appear to be normal. However, when we look at fully assembled complexes, using blue native electrophoresis, we observe a reduction in complexes I, III and IV. The detected reduction is not due to impaired mitochondrial translation as shown by in organello translation assays. We argue that point-mutations in the mitochondrial DNA alter the mitochondrially encoded respiratory chain subunits leading to failed assembly and ultimately reduced oxidative capacity.

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S14.5 Does aging influence lymphocyte mitochondria respiration in trained people?

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One of the major explanations for age-related decrease in general functionality of cell tissues has been the decline in mitochondria functionality. However, it have been stated that exercise induces an increase in mitochondria functionality. So, the aim of this study was to analyze the influence of exercise training on lymphocyte mitochondria function with aging. Fifteen men, aged between 19 and 52 years old (average age=32.6±10.6 years), engaged in regular physical activity participated in this study. Maximal aerobic capacity (VO₂max. ml kg⁻¹ min⁻¹) was assessed by spirometry until exhaustion. Lymphocyte mitochondria oxidative activity of Complex I and Complex II were assessed. A Pearson correlation was performed in order to test variables (age, mitochondria complex I and II respiration rate) associations. Significance level was established at 5%. VO₂max was 55.66 (+6.23) ml kg⁻¹ min⁻¹ and mitochondrial oxidative rate was 12.9±5.5 nmol oxygen/min/mg protein and 19.9±8.1 nmol oxygen/min/mg protein for Complex I and Complex II, respectively. Our results couldn't find any significant correlation between mitochondria oxidative rates and age. Concerning the high values obtained in maximal aerobic capacity of this sample and the lack of correlation between mitochondria oxidative capacity and age, we may conclude about the positive effects of exercise training in mitochondria functionality opposing the effect of aging process.

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S14.6 Impaired thermogenesis in PolgA mtDNA polymerase mutant mice

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Impaired thermogenesis is one of the features of ageing. Activity and recruitment of mitochondria in brown adipose tissue is important